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Comparable cortisol, heart rate and milk let-down in nurse sows and non-nurse sows

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Running title: Nurse sows and non-nurse sows

Abstract

Increasing litter size in hyperprolific sows has led to the need for management systems for surplus piglets, one of which is the use of nurse sows. The aim of this study was to investigate physiological changes in salivary cortisol, heart rate and number of milk let-downs in nurse sows compared to non-nurse sows. Sows were divided into three treatments: 1) control (non-nurse) sows nursed their own piglets until weaning at 26 days of age; 2) nurse1 sows had their own piglets removed and replaced with newborn piglets (between 6 - 24 h old) at Day 7, these were weaned at Day 33 of the sow's lactation period and 3) nurse2 sows weaned their own piglets at Day 21 and received a litter of 7 day old piglets from a nurse1 sow. These new piglets were weaned at Day 40 of the nurse2 sow's lactation period. Saliva samples were collected for cortisol analyses and the sows were fitted with pulse belts to monitor heart rate. Cameras were placed above the pens to record milk let-downs. Overall, there was no influence of treatment on salivary cortisol, heart rate or the number of milk let-downs/h. There was an effect of time as cortisol levels fell throughout lactation (P<0.001), and heart rate increased (P<0.001). Nurse1 sows had a lower cortisol concentration on Day 31 compared to Day 24 (P<0.028). The same was found for nurse2 sows, where the salivary cortisol concentration on Day 31 and Day 38 was significantly lower than on Day 24 (P<0.001). The present study found no differences in short-term (when the sows received new piglets) or long-term (throughout the lactation period) cortisol and heart rate measurements between different treatments. In addition, the frequency of milk let-down/h was the same for nurse sows as for non-nurse sows.

Key words: Cortisol, cross-fostering, heart rate, large litters, nurse sows

1. Introduction

In hyperprolific sows, litter size routinely exceeds the ability of individual sows to successfully rear all the piglets as viable piglets outnumber functional teats. Therefore, a number of management measures are being used. Nurse sows are widely used in countries such as Denmark and Holland with the two-step nurse sow system being the most common (Baxter et al. 2013). Nurse sows lactate for longer compared with non-nurse sows as they receive a “new” litter once their own piglets are weaned. As a result, the nurse sows stay in the farrowing crate for a longer period than she would have stayed if just rearing her own piglets. Recently, the welfare of nurse sows has been questioned and points of concern include the acute stress (behavioral and physiological) of removing the native litter and adopting a new one and the prolonged stress experienced by having to stay longer in farrowing crates (Baxter et al. 2013).

Acute effects of nurse sow systems are likely to include disputes at the udder due to a new litter which is likely to influence maternal behavior (Rutherford et al. 2013). In addition, a period of udder distension between removal and acceptance of litters might occur which is likely to cause discomfort as seen in dairy cows (Osterman and Redbo, 2000). It is also not known what the prolonged stay in the farrowing crates relates to in terms of sow welfare; a prolonged stay in farrowing crates with up to three weeks extra might cause changes in the hypothalamic-pituitary-adrenal (HPA) axis, suggesting chronic stress as reported by Jarvis et al. (2006).

The aim of this study was therefore to investigate the physiological response by measuring salivary cortisol, heart rate and the frequency of milk let-downs of nurse sows compared with non-nurse sows. We hypothesized that nurse sows would express acute (short-term) and prolonged (long-term) stress, measured by increased salivary cortisol concentrations, a higher heart rate and altered milk let-down frequency compared to non-nurse sows.

2. Material and methods

All sampling, housing and measurements were conducted in accordance with Danish laws and regulations for the humane care and use of animals in research [The Danish Ministry of Justice, Animal and Testing Act (consolidation Act no. 726 of September 9, 1993, as amended by Act No. 1081 of December 20, 1995)].

2.1 Animals and experimental design

This study was conducted on a Danish commercial piggery over two time periods (summer 2013 and winter 2013/14). Sixty-six sows of parity one to three (Danish Landrace × Danish Yorkshire) mated with Duroc semen (Hatting KS, Horsens, Denmark) were randomly allocated based on parity to one of three treatments on entry to the farrowing house at day 112 of gestation giving a total of 22 replicates per treatment. Treatment 1 was the control where sows kept their own piglets that were weaned 26 days after parturition (**non-nurse sows**). In treatment 2 (**nurse1**), the sow's own piglets were transferred at 1000 h to interim sows 7 days after parturition (4-8 days of lactation) and the sows received newborn 6 to 24 hour old piglets that were given direct access to the sow at 1015 h. These nurse piglets were weaned at Day 33 of nurse1 sow's lactation period, when the new piglets had an age of approx. 26 days. In treatment 3 (**nurse2**), the sows weaned their own piglets at Day 21 (21-24 days of lactation) at 1000 h and were then given an entire litter of 7 day old interim piglets from a nurse1 sow. These piglets were kept in the piglet creep area for 45 min to help facilitate milk let-down and let out at 1045 h. These interim piglets were weaned at Day 40 of the nurse2 sow's lactation period (approx. 26 days in piglet age) (see Figure 1). Piglets were always moved on Tuesday between 0945 and 1015 h.

Litters were standardized to 14 piglets, normally within 24 hours after birth, when it was assumed that all piglets had received colostrum. Day 0 was defined as the day the stock personnel would normally record the sows as having finished farrowing. Sows were selected with farrowing dates as close to each other as possible. A nurse1 or nurse2 sow did not receive more piglets than the number that was removed or weaned from her. Piglets were not moved again once they were allocated to a treatment group and sows were disturbed as little as possible. Piglets that died during the experimental period were not replaced and all sows were kept in the same section in the same pen throughout the trial. Piglets were neither castrated nor had their teeth ground. At 4 days of age, piglets were tail docked and given an injection of iron and an oral suspension of Baycox® 5% (Bayer, Germany). If sows showed signs of health problems they were excluded from the experiment and they were only replaced if this could be done prior to the Day 6 sampling. In order to facilitate swift sampling and moving of piglets; if more than 8 sows needed sampling, two experimenters performed the procedures together, and no more than three nurse sows were made on one day to ensure swift moving of piglets with three experimenters performing the task together.

Insert Figure 1somewhere around here

2.2 Housing and management routines

During gestation sows were kept in a loose housing system with straw. On day 106 of gestation they were moved to the farrowing section. The pens were conventional farrowing crates (Figure 2). Sows were fed a mash lactation diet three times a day (0700, 1130, 1500 h) according to Danish recommendations (Jørgensen, 2005) based on wheat (50.4%), barley (25%) and soybean meal (17%) as the main ingredients. The diet contained 7.9 MJ potential physiological energy/kg fed (Boisen, 2001) and 7.23 g standardized ileal digestible Lys/kg feed. The sows were given 3.9 kg prior to farrowing and 2.2 kg just before farrowing. After farrowing this amount was increased by 0.4 kg three times a week, and after one week, sows were fed to appetite with a maximum of 11.0 kg four weeks after farrowing. When receiving their new piglets on Day 21, nurse2 sows had their feed allowance down regulated to the amount distributed on Day 7. The feed curve of nurse1 sows was not modified when receiving their new piglets on Day 7. At 1530 h (half an hour before saliva sampling) the sows were given a handful of chopped straw as an environmental enrichment. In order to reflect a commercial farm situation nothing was done to limit disturbances from other pens etc and the farm followed normal routines. All sows were, however, kept in the same section and it was the same pens used throughout the trial (due to camera placement).

Insert Figure 2 somewhere around here

2.3 Recordings

Litter weight was recorded after litter equalization, and when nurse1 and nurse2 sows weaned or received new piglets as well as at normal weaning for non-nurse sows. Backfat depths at the P2 site were measured at week one and three for all sows and week five for nurse2 sows with a sono-grader (Renco, Minnesota, USA). Shoulder lesions were measured at weaning using a shoulder lesion score card (Kaiser and Petersen, 2014) giving a score of 0 for none or insignificant skin changes < 2 cm, a score of 1 for slight shoulder lesion > 2 cm and a score of 2 for a severe shoulder lesion > 5 cm.

2.4 Saliva samples

Saliva was collected from the sows at three time points (1000, 1300 and 1600 h) on Days 6, 8, 24, 31 and 38; and four times a day (0900, 1100, 1300 and 1600 h) on Day 7 and 21. These time points were chosen as cortisol follows a circadian pattern (Iranmanesh et al., 1989). A cotton swab (Salivette plain, Sarstedt, Leicester, UK) was attached to a surgical tong and placed in the mouth of the sows. Sows were allowed to chew on the cotton swab for approximately 30 seconds, or until saturated. Swabs were centrifuged at 1000 × g for 2 min at room temperature within half an hour of collection. The saliva was transferred to eppendorf tubes and stored at –20°C until analysis. For the short-term analysis, samples were analysed individually per time point but for the long-term analysis the three time points (1000, 1300 and 1600 h) were pooled before analysis. Saliva samples were assayed for cortisol levels in duplicate using a Salivary Cortisol EIA kit (Salimetrics, Newmarket, Suffolk, UK). Intra-assay variation was below 5 %, inter-assay variation was 6 % and were calculated using standards, and high and low controls from each plate. Cortisol concentration was quantified by interpolating absorbance readings from a standard curve generated in the same assay.

2.5 Heart rate measurements

To measure mean heart rate of the sows, pulse belts (model RS800CX, Polar Electro Oy, Finland) were placed around the chest of the sows on the mornings of Day 6 and Day 20, at least half an hour before the first saliva sample at 1000 h and removed after the final saliva sample of Day 8 and Day 22. Recordings were measured continuously from Monday to Wednesday (from the morning to late afternoon) but broken down into smaller time intervals for data handling and analysis. The pulse belts stayed on the sows from Monday until Wednesday afternoon and were adjusted during the experiment if needed. Specific timepoints (1000, 1300, 1600 and 1900 h) were chosen to compare mean heart rate, in a 5 min interval. The mean heart rate was defined as the amount of heartbeats per min. To correct for errors specific cut off points were chosen (i.e values below 0.2 and above 1) and the median of five, 5 min intervals were chosen within half an hour of the specific timepoint in question. Heart rate variability was calculated as the root mean square of successive differences (RMSSD) (data not shown).

2.6 Milk let-down observations

Video cameras (PTZ security IR-Dome model no. 795JH, PTZ Security, Esbjerg, Denmark) were placed above the sows in the farrowing unit. Behaviour was recorded from 0900 h on Day 6 until 2200 h on Day 8 for nurse1 and non-nurse sows and, between 0900 h on Day 20 and 2200 h on Day 22 for nurse2 and non-nurse sows. Behaviour was also recorded on day 24 for the three groups, on Day 31 for nurse1 and nurse2 sows, and on Day 38 for nurse2 sows. Video recordings were done using AxxonSoft software (AxxonSoft, Moscow, Russia) and extracted files were converted in OGG converter to jpeg files that could be read in a software programme developed by the Pig Research Centre for performing behavioral observations and registrations (RADRA, Pig Research Centre, Denmark) (Oxholm et al. 2014). The number of milk let-downs per hour was determined from 0900 h on Day 6 (the day before nurse1 received new piglets) until 2200 h on Day 8 (the day after nurse1 sows received new piglets) for the nurse1 sows and non-nurse sows and were recorded from 0900 h on Day 20 (the day before nurse2 received new piglets) until 2200 h on Day 22 (the day after nurse2 sows received new piglets) for the nurse2 sows and non-nurse sows. The ethogram used for registrations is presented in Table 1. The number of milk let-downs per hour included all behaviours presented in Table 1 except for unsuccessful suckling. It was observed that there could be cases of, for example, only 6 piglets from a total litter size of 14 that were present at the udder and the sow would still have a milk let-down. Therefore another category of a successful suckling (F) was included in the ethogram. For the analysis 10 hours per day were included.

Insert Table 1 somewhere around here

2.7 Statistical analyses

Treatment effects of becoming a nurse sow were estimated by fitting a linear-mixed model to salivary cortisol, heart rate, milk let-down, and production parameters and were analysed in SAS (MIXED procedure; SAS Inst. Inc., Cary, NC, USA). The short-term analyses of the effect of becoming a nurse sow were made according to the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \theta_{jk} + \varepsilon_{ijk}$$

Where Y_{ijk} is the dependent variable measured on the sow (salivary cortisol, heart rate), μ is an overall mean, α_i is the fixed effect of day (i = 6, 7, 8 or 20, 21, 22), β_j denotes the effect of time (j = 9, 10, 13, 16, 19), $(\alpha\beta)_{ij}$ is the interaction between day and time, θ_{jk} is the random effect of sow and ε_{ijk} the residual error. The repeated statement was included for time (day and hour) for the cortisol and heart rate analyses. When analysing data per hour the baseline measurement was included as a covariate (first cortisol sample 0900 h, and first heart rate 1000 h). No interaction between day and time was found and the variable was therefore removed from the final model.

For the effect of time on treatment the following model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \theta_{jk} + \varepsilon_{ijk}$$

Where Y_{ijk} is the dependent variable measured on the sow (salivary cortisol, heart rate), μ denotes the overall mean, α_i denotes the effect of treatment (i = non-nurse, nurse sow), β_j denotes the effect of time (j = 9, 10, 13, 16, 19), $(\alpha\beta)_{ij}$ is the interaction between treatment and time, θ_{jk} is the random effect of sow and ε_{ijk} describes the error term. The repeated statement was included for time (hour) for the cortisol and heart rate analyses. When analysing data per hour the baseline measurement was included as a covariate (as above). No interaction between treatment and time was found and the variable was therefore removed from the final model.

The long-term analyses were made according to the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \theta_{jk} + \varepsilon_{ijk}$$

Where Y_{ijk} is the dependent variable measured on the sow (salivary cortisol, heart rate and number of milk let-downs), μ denotes the overall mean, α_i denotes the effect of treatment (i = non-nurse, nurse1 and nurse2), β_j denotes the effect of time (j = day, hour), $(\alpha\beta)_{ij}$ is the interaction between treatment and time, θ_{jk} is the random effect of sow and ε_{ijk} describes the error term. The repeated statement was included for time (day and hour) for the cortisol analyses. No interaction between treatment and time was found and the variable was therefore removed from the final model.

For comparing individual salivary cortisol levels of nurse1 sows at Day 24 compared to Day 31, data of nurse1 sows was analysed using a t-test. A t-test was also used to compare nurse2 sows to themselves as Day 24, Day 31 and Day 38. For all parameters, the random and residual error components were assumed to be independent and normally distributed, and their expectations were assumed to be zero. Cortisol data were not normally distributed and therefore data were logarithmically transformed before analysis. Results presented for these data are therefore log transformed data with 95% confidence intervals given above the figures. Heart rate intervals were calculated over 5 mins, four times per day, starting 1005, 1305, 1605 and 1905 h, and were analysed in a model like the one used for cortisol. Video results were unbalanced and therefore calculated per hour, and the number milk let-downs per hour analysed in the same model. Means were separated using the PDIFF option and presented as LSMeans ± SE and considered significant when $P<0.05$ and a tendency when $P<0.10$.

3. Results

Short-term measurements were defined as differences over one or a few days whereas long-term measurements were defined as differences over the duration of the lactation period.

3.1 Production results

The production results can be seen in Table 2. There was no difference in sow parity, number of total born piglets, litter size after litter equalization, litter weight after equalization or backfat depth at the P2 site in week 1 (Table 2) between treatments. The first nurse1 litter weighed on average 33.3 ± 1.55 kg (7 day old piglets) and the first nurse2 litter on average 75.1 ± 2.51 kg (21 day old piglets). The new litter weight was on average 22.3 ± 1.26 kg for nurse1 sows (1 day old piglets) and 32.4 ± 1.59 kg for nurse2 sows (7 day old piglets). There was an effect of week on backfat depth at the P2 site with lower values towards the end of the lactation period (week 1; 14.9 ± 0.4 mm, week 3; 13.1 ± 0.4 mm and week 5; 12.3 ± 0.4 mm; $P<0.001$). Nurse2 sows lost 2.2 ± 0.4 mm backfat depth over the duration of the trial compared to non-nurse sows that lost 1.9 ± 0.4 mm and nurse1 sows that lost 1.7 ± 0.4 mm in back fat depth at the P2 site, however this difference was not significant (Table 2).

Insert Table 2 somewhere around here

3.2 Short-term salivary cortisol and heart rate

There was no short-term response to becoming a nurse1 sow (Figure 3A) and furthermore the heart rate was similar on Day 6 (before new piglets) and Day 7 (after new piglets) for nurse1 sows (Figure 3B). There was no difference in cortisol levels when comparing nurse1 sows with non-nurse sows (Figure 3C) or heart rates (Figure 3D) when nurse1 sows received new piglets at 1100 h on Day 7. There was an effect of time with heart rate values being highest at the afternoon (1600 h) reading ($P<0.05$) but no differences between treatments.

Insert Figure 3 somewhere around here

There was no short-term response on cortisol or heart rate to becoming a nurse2 sow (Figure 4A and B) with similar values on Day 20 (before new piglets) as on Day 21 (receiving new piglets) and Day 22 (after new piglets). Likewise, there was no difference in salivary cortisol levels (Figure 4C) or heart rates (Figure 4D) when comparing nurse2 sows with non-nurse sows when nurse2 sows received new piglets at 1100 h on Day 21. Day 22 salivary cortisol showed no differences (results very similar to Day 21) between nurse2 and non-nurse sows (data not shown). There was an effect of time on the heart rate response with significantly higher values being read in the afternoon (1600 h; $P=0.04$) compared to other time points, but there were no differences between treatments.

Insert Figure 4 somewhere around here

3.3 Long-term salivary cortisol and heart rate

Mean cortisol concentration or heart rate did not differ between treatments at any stage of lactation ($P>0.10$). Long-term salivary cortisol can be seen in Figure 5A. The long-term cortisol results showed that the nurse1 sows had lower cortisol concentrations on Day 31 compared to Day 24 ($P=0.028$). The same was found for nurse2 sows where the salivary cortisol concentration on Day 31 and Day 38 was lower than day 24 ($P=0.008$). The mean heart rate over 3 weeks can be seen in Figure 5B. There was an effect of time ($P<0.001$) with heart rate levels increasing during the lactation period for all treatment groups.

Insert Figure 5 somewhere around here

3.4 Milk let-down observations

Due to technical problems reading the video files from the second time period, some days and hours could not be analysed and were therefore excluded (specifically Day 24, 31 and 38 were very low in numbers n < 5 and therefore not included). The number of milk let-downs/h can be seen in Figure 6. There were no differences between treatments for days 6, 7, 8, 13 and 20. There was a tendency towards nurse2 sows having a higher frequency of milk let-down on Day 21 compared to nurse1 and non-nurse sows (P=0.060). On Day 22, nurse2 sows had more milk let-downs than nurse1 and non-nurse sows (P<0.002). However, when comparing the last milk let-down measurements where the piglets were the same age (approx. 24 days) and not the day of lactation for the sow the average number of milk let-downs was 1.8 milk let-downs/h for non-nurse sows at Day 24, 1.6 milk let-downs/h for nurse1 sows at Day 31 and 1.9 milk let-downs/h for nurse2 sows at Day 38 (n=5).

4. Discussion

Breeding programs towards hyperprolific sows have resulted in sows that produce a surplus of piglets compared to the sows number of functional teats (Baxter et al. 2013). This has created a demand for management options in order to increase production and welfare of the piglets. One management option is to create nurse sows using two-step nurse sow strategies as described in the study design. Recently, however, the welfare of these sows has been questioned as nurse sows have an extended lactation period and may spend up to 42 – 49 days in a farrowing crate not including the pre-farrowing period (Baxter et al. 2013). In addition, it has recently been reported that nurse sows have a significantly higher risk of swollen bursae on legs and udder wounds (Sørensen et al. 2016). In this study sows were chosen randomly. Stock personnel normally select high performing sows in good body condition as nurse sows (Sørensen et al. 1016) to ensure that they meet the demands of the new litter for care and milk. Thus maximum pressure was put on the nurse sows selected in the present study. Despite this only small differences in physiological responses and milk let-downs were found between the different groups in this study.

Stressful experiences stimulate the synthesis and release of glucocorticoids (Francis and Meaney, 1999), and salivary cortisol can be an effective and accurate tool for assessing stress if data are interpreted correctly acknowledging the circadian rhythm and the physiological state of the animal (Hawkins et al. 2014), and if the sampling method itself is not deemed a stressful experience. In addition, cortisol production shows large inter-individual variation which has a considerable genetic basis (Murani et al. 2012) and this should therefore also be considered. Differences in short term cortisol concentrations in response to stressful experiences have been shown in relation to mixing of gilts during pregnancy (Couret et al. 2009) and to loading and journey type (rough versus smooth) (Bradshaw et al. 1996). Furthermore, castration in pigs increases cortisol concentrations (Prunier et al. 2005) and feeding gilts restrictedly causes a significant increase in morning cortisol levels compared to gilts fed a high feed level in salivary cortisol (Amdi et al. 2013). In the current study no difference was found in the short-term salivary cortisol levels between the nurse and the non-nurse sow. Hence the results suggest that receiving new piglets during lactation did not evoke a measurable physiological stress response by saliva sampling. A more accurate measurement, for example through continuous blood sampling at the specific time point when the sows received the new piglets would perhaps have shown a more sensitive and accurate result, albeit more invasive and challenging to perform under commercial conditions.

The mean salivary cortisol of both nurse sows and non-nurse sows fell over lactation, reaching levels as low as 11.1 nmol/l at Day 24 and 7.4 nmol/l at Day 38 of lactation. Shortly after a stress has begun the hypothalamic-pituitary-adrenal (HPA) axis may become activated resulting in elevated cortisol output, however over time the body could mount a counter–

regulatory response that ensures that cortisol output rebounds below normal (Miller et al. 2007). A prolonged stay in farrowing crates with up to three weeks extra might cause changes and an adaption in the HPA axis suggesting chronic stress as found by Jarvis et al. (2006) on sows that had been confined up to 29 days in farrowing crates after a corticotrophin releasing hormone (CRH) challenge. In addition, a study by van der Staay et al. (2010) comparing the effects of chronic stress in tethered and loose sows, found that chronically stressed sows develop depression-like symptoms measured by the size of the pituitary gland and differences in for example β-globin mRNA in the hippocampus. Thereby suggesting that recurring stress over 4.5 years had lasting neuroendocrine effects (van der Staay et al. 2010). However, as both non-nurse and nurse sows reached very low levels over the same period of time in this study, it is unlikely that being selected as a nurse sow will affect salivary cortisol concentration in a hormone challenge such as a CRH challenge. In addition, nurse2 sows had significantly lower values when compared to themselves on Day 31 and Day 38 compared to Day 24 suggesting a physiological decrease in cortisol as lactation progresses. Therefore, it is important not only to conclude on the stress level by investigating cortisol levels, other factors must be measured to make more definitive conclusions. Whilst heart rate and the occurrence of milk let-downs/h are not exhaustive measures of behavioural disturbance they do give the ability to inform us further about the sow's physiological state.

The heart rate showed a significant effect of time, with values varying over the day and also over the duration of the lactation period with higher values towards the end of the lactation period. Although heart rate only shows the net effect of the vagus (Rietmann et al. 2004), heart rate can vary according to the body's physical needs which can be changed by stress (Kudielka et al. 2004). The afternoon heart rate was highest for all groups. These findings could be explained by a general higher activity level in the afternoon, which also coincided with the sows receiving their afternoon straw. The increasing heart rate throughout the lactation period could be explained by the increasing blood flow needed for milk production (Farmer et al. 2008). Short-term and long-term heart rate did not differ between non-nurse, nurse1 and nurse2 sows and therefore not influenced by nurse sows (both 1 and 2) receiving new piglets and can thus be attributed to normal physiological responses as the two mentioned (i.e. general higher activity level in afternoon and increasing blood flow).

The prolonged time nurse sows remain confined in farrowing crates raises welfare concerns, and it was hypothesised that this would change the nursing behavior of the nurse sows compared to non-nurse sows. For example, the nurse sow (nurse2) receiving 7 day old piglets after weaning her own 21 day piglets might experience a build-up of milk when not suckled at normal intervals (Baxter et al. 2013). Also, cross-fostering disrupts the teat suckling relationships of the whole litter compared with those of non-nurse litters and resulted in sows snapping more at fostered piglets than non-nurse sows did (Robert and Martineau, 2001). In the current study, the nurse2 sows quickly adjusted to the new piglets, a possible explanation for this could be that entire litters were transferred rather than in a normal cross-fostering protocol where a proportion of piglets already established on the udder remain with others being fostered on. In addition, nurse2 sows tended to have higher frequencies of milk let-downs on Day 21 and significantly more on Day 22 compared to nurse1 and non-nurse sows. This can however be explained by the piglets not being the same age at Day 21 (nurse2 received 7 day old piglets) and can therefore not be compared without taking into account the age of the piglets. It does, however, reject the hypothesis that nurse2 sows would experience more unsuccessful milk let-downs. One of the methods of making sure the sow will accept a new litter is by keeping the piglets in the creep area for an hour in order to let the sow accumulate milk (English, 1999). This was therefore the procedure used for the nurse2 sows that received the 7 day old piglets and could also explain the quick adoption of the new litter. In agreement with the current study, Heim et al. (2012) found that there was no adverse effects on growth performance of piglets that were cross-fostered and they found no differences in percentage of missing nursing periods. The number of milk let-downs when the piglets were the same age (approx. 24 days) rather than the day of lactation for the sow showed similar results suggesting that the age of the piglet influences milk let-downs.

In the wild, the proportion of sucklings initiated by the sow decreased after 10 weeks of lactation with an average weaning age of 17.2 weeks (Jensen and Recén, 1989). Therefore it could be argued in terms of the biology of the pig that it is not an unusually long lactation time that the nurse2 sow experienced. However, the sows studied by Jensen and Recén (1989) suckled fewer (on average 50% less) piglets than sows in the current study (avg 7.4 piglets, range 5-10), the piglets could forage and the sows were not under close confinement. Hence in this respect nurse2 sows in the current study experienced greater challenges. It is possible that sows can adapt very quickly to the new situation (Baxter, 1989). However, the possibility that this is a coping mechanism in response to apathy (van der Staay et al. 2010) should also be considered. In addition, crated sows are less able to respond, and thus behavioural as well as physiological responsiveness to environmental challenges may be downregulated (von Borell et al. 2001).

A prolonged lactation might lead to loss of body condition and shoulder ulcers (Rutherford et al. 2013), as well as claw and other limb lesions, thereby causing discomfort to the sow (Larsen et al. 2015). In the current study neither the nurse1 or nurse2 sows developed shoulder ulcers despite losing 2 mm in P2 back fat and no differences were found between treatments. This corresponds with a recent study by Sørensen et al. (2016), who found no difference in the occurrence of shoulder lesions between nurse and non-nurse sows. Sørensen et al. (2016) did however report an increased risk of swollen bursae on legs. But besides, Sørensen et al. (2016), limited research is available of the impact of confinement for longer periods on for example claw health. In addition the focus of the current study was on the sow and further studies on the effect on the piglets on being moved to a nurse sow are warranted.

5. Conclusion

Nurse sows spend up to 45 days in a crate; however this current study found no differences in short-term or long-term cortisol and heart rate measurements between different treatments. In addition the amount of milk let-downs/h was the same for the nurse sow towards a new litter as for sows nursing their own piglets. It cannot be ruled out that the non-significant findings throughout the experiment could be due to the lack of sensitivity of the on-farm measurements.

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Table 1. Ethogram of the sow's milk let-down behaviour

Behaviour	Description
Start of suckling (D)	Suckling defined as more than 75 % of the litter being active at massaging the udder and suckling bout sees piglets in brace position attached to functional teats and completing a suckling.
Successful sucklings (S)	50 % or more of the piglets are active at the udder (within one piglet length of the udder, with sow lying laterally) 2 minutes or more, prior to milk let down.
Unsuccessful sucklings (I)	Sow terminates or piglets terminate before milk let-down
Successful suckling (F)	Less than 50 % of the piglets are active at the udder (within one piglet length of the udder, with sow lying laterally) 2 minutes or more, prior to milk let-down.
Unsure (U)	If unsure of an actual milk let-down (piglets at udder but not suckling) U was recorded.

431 **Table 2.** Production results and backfat depth at the P2 site for non-nurse, nurse1 and nurse2
432 sows

	Treatment				*P-values*
	Non-nurse	Nurse1	Nurse2	SE	
n	24	23	22		
Parity	1.8	1.8	1.6	0.15	0.48
Total born	18.5	17.5	17.2	0.70	0.64
Littersize after equalisation	14.0	13.9	14.0	0.04	0.35
Litterweight after equalisation	19.9	19.6	20.4	0.49	0.37
Day of lactation when piglets were moved	na[1]	6.9	21.6	0.36	na
No of piglets moved	Na	12.9	12.7	0.22	na
Average number of piglets received by sows	Na	12.9	12.4	0.20	na
Lactation days at weaning	24.6	31.9	39.6	0.37	na
Litterweight at weaning	85.0	73.8	73.9	0.37	na
Total weaned	13.0	11.1	12.0	0.25	na
Backfat P2, mm					
Week 1	14.8	15.1	14.0	0.37	0.38
Week 3	13.0	13.4	12.7	0.37	0.68
Week 5	Na	na	11.8	0.42	na
Backfat loss P2,mm	1.9	1.7	2.2	0.4	0.37

433 [1]Results are non-comparable due to time differences and therefore there are no *P*-values for
434 some variables. The SE presented are pooled SE values across treatments.

Fig. 1. The timeline of the nurse sow trial. The black arrow shows the timeline - all times are related to the length of time the sows are lactating after farrowing – not piglet age. The grey arrows show the time the sow spends with her own piglets. The broken arrow indicates piglets being crossfostered from nurse1 to nurse2 sows. Non-nurse sows weans piglets at 26 days, nurse1 sows weans at 33 days and nurse2 sows wean their final batch of piglets at 40 days.

Fig. 2. Layout of the pen all sows were kept in during the lactation period.

Fig. 3. A) Short term salivary cortisol of nurse1 compared to herself at 1000, 1300 and 1600 h on Day 6, Day 7 and Day 8 and B) average heart rates of nurse1 compared to herself at 1000, 1300, 1600 and 1900 h on Day 6 compared to Day 7 and C) salivary cortisol of nurse1 compared to non-nurse sows at 0900, 1100, 1300 and 1600 on Day 7 (new piglets) and D) heart rates of nurse1 compared to non-nurse sows on Day 7 (new piglets) at 1000, 1300, 1600 and 1900 h. The salivary cortisol data that are presented here are log transformed with normal scale (arithmetic) backtransformed values in brackets. Heart rate values presented are means ± pooled SE. Letters denote effect of time.

Fig. 4. A) Short term salivary cortisol of nurse2 compared to herself at 1000, 1300 and 1600 h on Day 20, Day 21 and Day 22 and B) average heart rates of nurse2 compared to herself at 1000, 1300, 1600 and 1900 h on Day 20 compared to Day 21 and C) salivary cortisol of nurse2 compared to non-nurse sows at 0900, 1100, 1300 and 1600 on Day 21 (new piglets) and D) heart rates of nurse2 compared to non-nurse sows on Day 21 (new piglets) at 1000, 1300, 1600 and 1900 h. The salivary cortisol data that are presented here are log transformed

with normal scale (arithmetic) backtransformed values in brackets. There was an effect of time *(P < 0.05). Heart rate values presented are means ± pooled SE

Fig. 5. Long-term salivary cortisol and heart rates in non-nurse, nurse 1 and nurse 2 sows A) Pooled salivary cortisol levels over days and B) average heart rates. The cortisol data that are presented here are on a normal scale (arithmetic) backtransformed values. Sows differed significantly on day *(P < 0.05), **(P < 0.01) when compared to themselves. Letters denote effect of time. The salivary cortisol data that are presented here are log transformed with normal scale (arithmetic) backtransformed values in brackets. Heart rate values presented are means ± pooled SE

Fig. 6. Milk let-downs/h for non-nurse, nurse1 and nurse2 sows. Treatments differed significantly on day *(P < 0.05), **(P < 0.01), ***(P < 0.001). Values presented are means ± pooled SE. Day 6 (n = 17, 22, 13), Day 7 (n = 17, 21, 11), Day 8 (n = 10, 10, 10), Day 13 (n = 13, 14, 6), Day 20 (n = 17, 17, 21), Day 21 (n = 18, 10, 21) and Day 22 (n = 9, 10, 10) for non-nurse, nurse1 and nurse2 sows, respectively.

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